Amyloid Fibrils Formed by Peptide Sequences from a Natural β-Structured Fibrous Protein, the Fibre of Adenovirus

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Introduction

Natural fibrous proteins such as silk fibroins, spider silks and viral spikes are generally both stable and flexible and have recently been shown to contain novel β-motifs composed of repetitive structural elements [1]. Our work focuses on the study of folding, structure and assembly of such a β-structured protein using the fibre from adenovirus as a model system. Adenovirus fibres are trimeric proteins protruding from the viral capsid; they consist of three segments: an N-terminal tail, a long shaft, and a C-terminal globular head. The shaft contains a repeating sequence motif of 15 residues with an invariant glycine or proline and a conserved pattern of hydrophobic amino acids. The structure of a stable domain, comprising the globular head and four shaft repeats [2], has previously been solved at 2.4 Å resolution, revealing a novel triple β-spiral fibrous fold for the shaft [3]. The repeating motif of 15 residues comprises an extended β-strand which runs parallel to the fibre axis, followed by a β-turn containing the conserved glycine or proline. The turn is followed by another β-strand which runs backwards and forms an angle of 45° with the shaft axis; a solvent-exposed loop connects this strand with the extended one of the next repeat. The globular head is thought to play an essential role in the assembly of the protein as mutations or deletions in this part inhibit trimerisation [4, 5]. In order to study the folding and assembly mechanisms of shaft sequences in the absence of the head, a 41 amino-acid peptide corresponding to the part of the shaft that is immediately adjacent to the head was synthesised and studied. This peptide failed to fold into its native triple β-spiral conformation; instead, it self-assembled into amyloid-type fibrils [6]. Amyloid fibrils usually form as a result of misfolding events in proteins, and are associated with diseases such as Alzheimer's and prion related diseases[7]. In an effort to investigate whether amyloid fibril formation is a general propensity of the shaft sequences, we synthesised shorter synthetic peptides (25, 12, 8, 6 and 4 amino acids) that correspond to specific repetitive sequences from the adenovirus fibre shaft [8]. We report here on the structure and assembly of these 6 peptides using a combination of fibre diffraction, infrared spectroscopy, Congo Red binding and electron microscopy approaches.

Experimental procedure

Peptide synthesis: C-terminal amide derivatives of the 41-mer, 25-mer, 12-mer and 4-mer peptides were synthesised using in-house solid phase chemistry on the Applied Biosystems 430A automated peptide synthesizer [6]. The 6-mer and 8-mer peptides were purchased from Bachem, France. The purity and identity of the peptides were assessed by reverse-phase high performance liquid chromatography and electrospray mass spectrometry. Peptide concentrations were determined by in-house amino acid composition analysis.

Electron microscopy: Peptide powders were dissolved in water prior to adsorption to carbon-coated copper grids. The concentration of the solutions ranged from 0.5 to 20 mg/ml depending on the peptide. The samples were stained with 1% sodium silicotungstate and allowed to dry. Grids were examined with a JEOL 1200 XII transmission electron microscope. Negatively stained crystals of catalase were used as a calibration standard.

Congo Red staining and Birefrigence: Peptide fibril suspensions were stainded with a 10 μM Congo red solution

in water. The fibrils were concentrated by centrifugation and applied to glass slides. Samples were observed with a Zeiss Axiophot microscope equipped with cross-polars.

Fourier Transform Infra Red Spectroscopy (FTIR): FTIR samples were prepared by dissolving the peptides in D_2O except for the 25 amino acid peptide which was dissolved in 10mM deuterated ammonium acetate buffer pH 4. Samples were placed between two CaF_2 windows (Spectra tech) separated by a 100 µm spacer. All spectra were recorded on a JASCO 610 Fourier transform spectrometer with a 4 cm^{-1} resolution at room temperature. The interferograms from sixteen scans were averaged, and the solvent spectrum was subtracted.

X-ray fibre diffraction: Lyophilised peptide powders were dissolved in 10mM ammonium acetate buffer pH 4. Samples for X-ray fibre diffraction were prepared by air-drying a 5 µl drop of fibril solution between two glass-rod ends. For the 41 amino acid peptide, alignment of the fibrils was improved by the use of the magnetic field available in the NMR laboratory at the Institut de Biologie Structurale in Grenoble. For the peptides of 25 and 12 amino acids, we did not note any improvement in the alignment by the use of magnetic field.

Samples were studied using the microfocus beamline ID13 at the European Synchrotron Radiation Facility (ESRF), equiped with a MAR CCD detector with a 130 nm entrance window and the following readout parameters: 2048 x 2048 pixels, 64.45 x 64.45 µm pixel size. The wavelength was 0.976 Å and the beam was 5-10 µm in diameter. The sample-to-detector distance ranged from 57.1 - 42.8 mm. Diffraction datasets were processed and measured using CCP13 software [9] and the FIT2D package [10].

Results and Discussion

The design of different peptides was based on the crystalline structure of the native protein (Fig. 1). The 4 amino acid peptide corresponds to the flexible region connecting the head to the shaft and is not part of the shaft repeats.

Amyloid is defined by three criteria, namely its tinctorial affinity for the dye Congo Red, its fibrillar morphology upon analysis by electron microscopy and its characteristic "cross-β" X-ray diffraction pattern. Linear, non-branching fibrils with variable lengths and a diameter ranging from 16 to 30 Å have been detected by electron microscopy (Fig. 2a) for all the peptides except for the 4 amino acid one. These fibrils bind Congo Red and produce a gold-green birefringence under polarised light (Fig. 2b). Infrared results (Fig. 3) suggest that the fibrils formed from the different peptides adopt a β-sheet conformation as revealed by the presence of the band at ~1620 cm^{-1}. The spectrum recorded for the 4 amino acid peptide displays a peak at 1645 cm^{-1} characteristic of the random coil conformation.
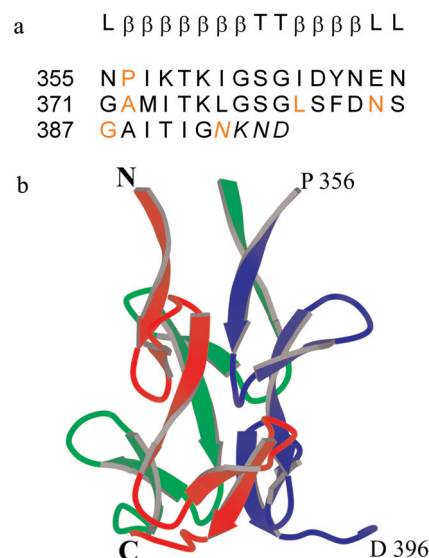

Figure 1: a) Sequence of the various peptides. Residue numbers correspond to positions in the full-length fiber sequence. Each line corresponds to a sequence pseudo-repeat of 15 residues. The last four amino acids in italics are not part of a repeat but belong to the linker region that connects the shaft to the head domain. The first amino acid of each peptide is coloured. The conserved secondary structure of the repeats is shown above with L for loop, β for β-strand and T for β-turn. b) Structure of the 41 amino acid peptide within the native shaft segment. The three chains are coloured differently; their N and C-termini are labeled corresponding to residues 355 and 396 respectively. All the other peptides are subsequences of the 41-mer. (Figure courtesy of Dr. Mark van Raaij, Universidad de Santiago de Compostela, Spain).

Fibre diffraction has turned out to be a key technique for the study of amyloid structures The power of the method lies in the fact that the data produced are usually directly relevant to the system being studied, and less affected by artefacts that can be important in single crystal work (e.g limited length of oligomers, presence of crystallising agents). The current diffraction work was aimed principally at using fibre diffraction as a means identifying the fibres as amyloid in character. The samples produced as described above were found to be cylindrically symmetric -i.e. producing diffraction patterns that were invariant as a function of rotation about the fibre axis. Figure 4 shows typical "cross-β" X-ray fibre diffraction patterns recorded from the 41, 25, 12, 8 and 6 amino acid peptides. A clear meridional reflection at 4.7 Å and a more diffuse reflection on the equator between 10 and 11 Å are present in all five patterns. These two reflections are consistent with a β-structure where the β-sheets are spaced by ~10 Å and arranged parallel to the fibre axis with their β-strands aligned perpendicular to that direction [11, 12]. The 4.7 Å reflection on the meridian corresponds to the interstrand distance. Further reflections in the meridional direction at ~2.4 Å and ~1.6 Å are visible in all patterns as shown for the 25 amino acid peptide in Fig. 5. The
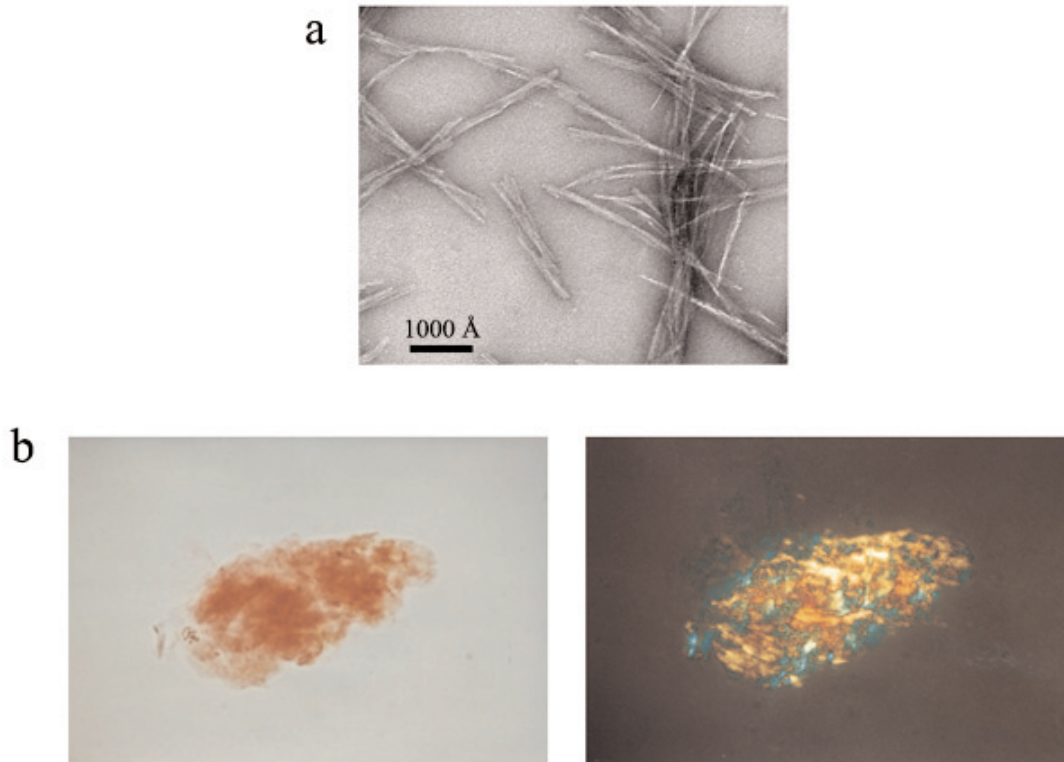
Figure 2: Results are shown for the 41 amino acid peptide. a) Electron micrograph of fibrils stained with 1% sodium silicotungstate. The peptide was solubilised in water at a 0.5 mg/ml concentration. b) Photomicrographs of fibrils stained with Congo Red and observed under bright field illumination (left pannel) and between crossed polars (right pannel). Similar observations were made for all of the five amyloid-forming peptides.

equatorial reflections in the patterns varied very significantly; they are diffuse in the pattern of the 41 amino acid peptide and become more distinct in the shorter peptides. As judged by the above structural criteria, all the peptides, except for the 4-mer, have an intrinsic capacity to form amyloid fibrils. Thus, self-assembly into amyloid fibrils is a general property of the shaft sequences when isolated from their native context. The X-ray fibre diffraction and infrared results suggest that the fibrils formed from the different peptides share a common β-sheet framework structure where the β-strands lie perpendicular to the fibre axis. From the crystal structure of the shaft segment and the current structural studies it becomes obvious that the amyloid signature is different from the native triple β-spiral fold. The above results further support the essential role of the head domain in fibre assem-

Figure 3: Infrared spectra of the peptide solutions made from the a) 41-mer b) 25-mer c) 12-mer d) 8-mer e) 6-mer and f) 4-mer. Only the amide I region is shown. The positions assigned to the various secondary structure elements are marked as RC for random coil and β for β-sheet. The position corresponding to residual trifluoroacetic acid as a result of the purification procedure is marked as TFA. Peptide solutions were prepared and spectra were recorded after 44 hours for the 41 aa peptide, 18 hours for the 25 aa, 32 hours for the 12 aa, 20 hours for the 8 aa, 6 days for the 6 aa and one month for the 4 aa peptide. The concentrations of the peptide solutions were as follows: 10 mg/ml for the 41-mer, 25-mer and 4-mer, 5 mg/ml for the 12-mer and 20 mg/ml for the 8-mer and 6-mer.
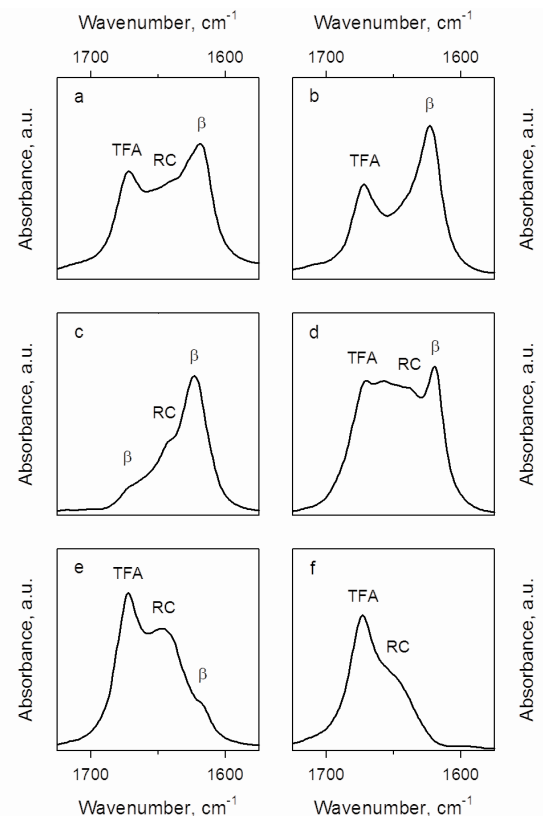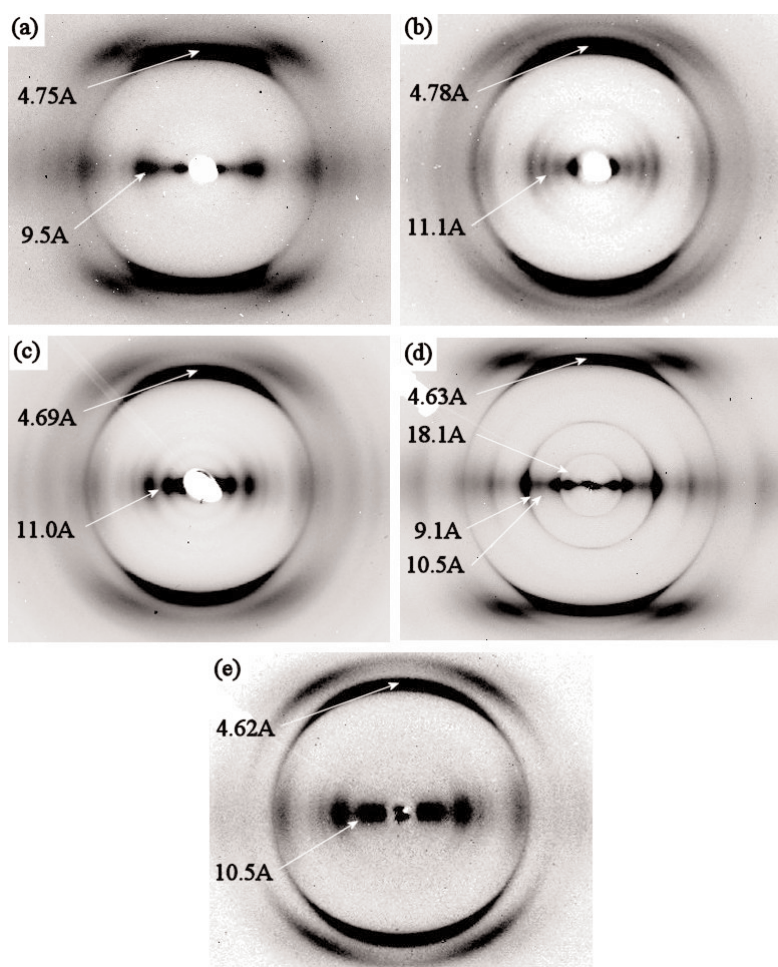
Figure 4: Diffraction patterns recorded from amyloid fibres formed from the shaft peptides of a) 41 b) 25 c) 12 d) 8 and e) 6 amino acids. The two major characteristic reflections (4.7 Å and ~10 Å) of the cross-β structure are marked with arrows. In the pattern of the 8-mer two additionnal reflections at 9.1 Å and 18.1 Å are indicated.

bly. The aggregation of the shaft sequences into amyloid may occur as a result of out-of register interactions in the absence of the head. The globular part might act as a registration signal necessary for the three chains to align and fold together. It is worth mentioning that, when the native head is replaced by a foreign trimerisation motif, the shaft sequences trimerise successfully and adopt the triple β-spiral conformation [13, 14]. The results reported in this paper are relevant to understanding amyloid formation by repetitive sequences from disease-associated proteins. The adenovirus shaft sequences can provide a model system to study folding, assembly and registration of β-type structures both in native and in amyloid contexts.
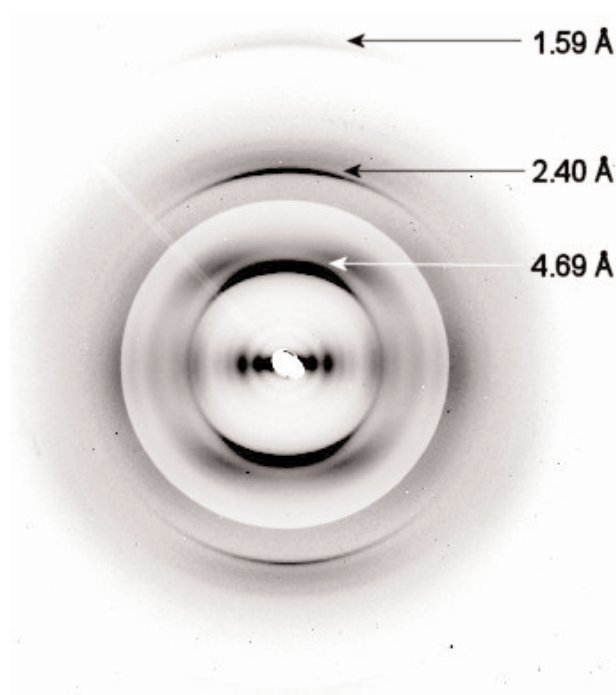

Figure 5: X-ray fibre diffraction pattern taken from aligned fibrils of the 12-mer. The observed meridional reflections at 1.59 Å and 2.40 Å as well as at 4.69 Å are indicated with arrows. Similar results were obtained for all the other peptides.

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